EFFECT OF LYSINE MODIFICATION ON THE CONFORMATIONAL STABILITY OF α-AMYLASE

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ABSTRACT

Chemical modifications of *Bacillus licheniformis* α–amylase (BLA) were carried out using 100 molar excess of succinic anhydride over protein, 0.66 M potassium cyanate and 0.46 M O-methylisourea under standard conditions to prepare different modified derivatives. The percentage of modification as determined by TNBSA reaction method was found to be 42% for succinylated (S_{42}), 81% for carbamylated (C_{81}) and 88% for guanidinated (G_{88}) BLA derivatives. These modified preparations were checked for their size and charge homogeneity by gel chromatography on Sephacryl S-200 HR column and polyacrylamide gel electrophoresis. Conformational changes in these preparations were detected by analytical gel chromatography on a calibrated Sephacryl S-200 HR column by determining the Stokes radii of native and modified derivatives. C_{81} BLA was found to have the highest Stokes radius value followed by S_{42} and G_{88} BLAs. Conformational stabilities of native and modified BLA derivatives were investigated by urea denaturation using circular dichroism (CD) spectroscopy. Denaturation profiles showed a decrease in the stability of modified BLAs being more pronounced in C_{81} BLA. In addition, the effect of calcium on urea and guanidine hydrochloride (GdnHCl) denaturation of native and calcium-depleted enzymes was also analyzed by CD, fluorescence spectroscopy and biological activity. Based on these results, calcium was found to stabilize the enzyme against both urea and GdnHCl denaturations.

ABSTRAK

Modifikasi kimia digunakan untuk *Bacillus licheniformis* α–amilase (BLA) dengan menggunakan 100 molar ekses suksinik anhidrida melebihi protein, 0.66 M kalium sianat dan 0.46 M O-metilisourea dalam kondisi tetap untuk menyediakan pelbagai derivatif modifikasi. Peratus modifikasi ditentukan melalui tindak balas protein dengan TNBSA dan ditetapkan sebagai 42% untuk suksinilasi (S_{42}), 81% untuk karbamilasi (C_{81}) dan 88% untuk guanidinasi (G_{88}) BLA. Ketulenan dari segi saiz dan cas untuk semua protein yang telah melalui process modifikasi ditentukan dengan kaedah gel kromatografi dengan manggunakan kolum Sephacryl S-200 HR dan poliakrilamida gel electrophoresis. Perubahan konformasi dalam penyediaan tersebut pula ditentukan dengan analitikal gel kromatografi atas kolum Sephacryl S-200 HR yang telah terkalibrat untuk menentukan jejari Stokes. C_{81} BLA didapati mempunyai jejari Stokes yang paling tinggi diikuti dengan S_{42} dan G_{88} BLA. Kestabilan konformasi untuk protein asli dan derivative modifikasi disiasat dengan denaturasi urea menggunakan 'circular dichroism' (CD) spektroskopi. Profil denaturasi protein-protein tersebut menunjukkan pengurangan kestabilan dalam protein yang telah termodifikasikan terutamanya dalam penyediaan C_{81} BLA. Selain itu, impak kalsium atas urea dan GdnHCl denaturasi protein asli dan BLA di mana intrinsik kalsium yang telah dikeluarkan ('Ca-depleted BLA') juga dianalisa dengan mengunakan CD, 'fluorescence' spektroskopi dan aktiviti biologi. Berdasarkan keputusan tersebut, kalsium ditemui sebagai ejen penstabil BLA daripada urea dan GdnHCl denaturasi.

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ANS	8-Anilino-1-napthalene sulfonic acid
BAA	*Bacillus amyloliquefaciens* α-amylase
BLA	*Bacillus licheniformis* α–amylase
BSA	Bovine serum albumin
BStA	*Bacillus stearothermophilus* α-amylase
BSUA	*Bacillus subtilis* α-amylase
c	Protein concentration in mg/ml
°C	Degree Celsius
C_{81}	81% Carbamylated
Ca	Calcium
$CaCl_2$	Calcium chloride
cal/mol/M	Calories/mole/molar
CD	Circular dichroism
cm	Centimeter
[D]	Denaturant concentration
deg.	Degree
dmol	Decimole
DMSO	Dimethyl sulfoxide
DNS	Dinitrosalicylic acid
e.g.	Latin phrase *exempli gratia* (for example)
EGTA	Ethylene glycol-bis (2-aminoethyl-ether)-N,N,N',N'-tetraacetic acid
etc	Latin phrase *et cetera* (and so on)
F_D	Fraction denatured
g	Gram
G_{88}	88% Guanidinated
ΔG_D	Free energy change
$\Delta G_D^{H_2O}$	Free energy of stabilization
GdnHCl	Guanidine hydrochloride
Glu	Glutamic acid
h	Hour
h	Height
HCl	Hydrochloric acid
i.e.	Latin phrase *id est* (that is)
K_{av}	Available distribution coefficient
K_D	Equilibrium constant
K_d	Distribution coefficient
l	optical path length of the cell
Lys	Lysine
M	Molar
m	Slope value
m	Measure of the dependence of ΔG_D on denaturant concentration
mg	Milligram
min	Minute
ml	Milliliter
mM	Millimolar
mm	Millimeter
MRE	Mean residue ellipticity
$MRE_{222\ nm}$	Mean residue ellipticity at 222 nm
MRW	Mean residue weight

NaCl	Sodium chloride
NaOH	Sodium hydroxide
No.	Number
nm	Nanometer
PAGE	Polyacrylamide gel electrophoresis
Phe	Phenylalanine
R	Gas constant
r	Radius
S_{42}	42% Succinylated
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate- polyacrylamide gel electrophoresis
SmF	Submerged fermentation
SSF	Solid-state fermentation
T	Absolute temperature
TEMED	N, N, N', N'-Tetramethylethylenediamine
T_m	Melting temperature
TNBSA	Trinitrobenzenesulfonic acid
Tris-HCl	Tris(hydroxymethyl)aminomethane-hydrochloride
Trp	Tryptophan
Tyr	Tyrosine
µg	Microgram
µM	Micromolar
UV	Ultraviolet
V	Volume
V	Voltage
V_i	Inner volume
V_o	Void volume
V_t	Total volume
v/v	Volume per volume
w/v	Weight per volume
Y	Variable parameter
Y_D	Variable parameter for the denatured state
Y_N	Variable parameter for the native state
θ_{obs}	Observed ellipticity
~	Approximate
≥	Greater than or equal to
%	Percentage
±	Plus-minus